TISSUE CULTURE AND DROUGHT RESISTANCE OF CHICKPEA (*Cicer arietinum* L.)

by

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Dedicated to my mother Latifa and

to the memory of my father Mokhtar

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PART I

TISSUE CULTURE OF CHICKPEA (*Cicer arietinum* L.)

ABSTRACT

Tissue culture techniques were investigated using chickpea (Cicer arietinum L.) to ascertain requirements for callus formation and plant regeneration. Two media were used, DM1 and B5-1, to initiate callus from six cultivars: NEC1163, ILC202, ILC262, ILC482, ILC511, and Local (from Tunisia). Calli were produced mainly on the B5-1 medium from three cultivars: ILC262, NEC1163, and Local. Eight media were used (B5a, B5b, B5c, B5d, B5e, B5f, B5g, and B5h) to determine the suitability of growing calli from these three cultivars. The average callus fresh weight of the three cultivars was 0.188 g on B5c containing 1 mg of BAP/l and 0.5 mg of NAA/l, whereas the average callus fresh weight was only 0.083 g on B5g (4 mg of 2,4-D/l and 1 mg of kinetin/l). Among the three cultivars, ILC262 had the highest mean fresh weight of callus (0.152 g) over time on all media. The two other cultivars (NEC1163 and Local) did not differ significantly from each other (0.094 g and 0.115 g, respectively).

Three media were used for plant regeneration from calli (B5i, B5j, and DMG1). None of the media used for regeneration gave satisfactory results. Only root formation took place from leaf explants on B5f containing 2 mg of 2,4-D/l and 1 mg of kinetin/l which was used for callus growth.

INTRODUCTION

In vitro methods help plant breeders conduct basic genetic research on higher plants at the cellular level. They are potentially powerful new tools to generate, select, and propagate novel and economically important crop cultivars. It is now possible to achieve rapid clonal propagation of plants, especially herbaceous species, by tissue culture and to attempt new combinations of genes for genetic improvement. Two basic requirements for the application of tissue culture are the establishment of callus tissue (i.e., induction of cell proliferation from explants or from single cells) and regeneration of intact plants from callus tissues.

Chickpea (*Cicer arietinum* L.), a legume, is an important crop in the world. Yet little information exists concerning its response and development in tissue culture. The present study was conducted to determine the suitable medium for callus induction and for plant regeneration.

LITERATURE REVIEW

A few leguminous species have been regenerated successfully by using callus cultures. Among them are alfalfa (Medicago sativa L.) (Saunders and Bingham 1972, 1975), pea (Pisum sativum L.) (Gamborg et al. 1974), Stylosanthes hamata (Scowcroft and Adamson 1976), grass pea (Lathyrus sativus) (Mukhopadhyay et al. 1980), red clover (Trifolium pratense) (Horvath and Smith 1979), sesban (Sesbania sesban) (Khattar and Mohanram 1982), and birdsfoot trefoil (Lotus corniculatus L.) (Swanson and Tomas 1980).

Carew and Shwarting (1958) reported the successful initiation and maintenance of callus from immature rye (Secale cereale L.) embryos on medium containing 2,4-dichlorophenoxyacetic acid (2,4-D). In oats (Avena sativa L.), callus was induced from germinating whole seeds with maximum callus development occurred on media containing 2,4-D (Webster 1966; Carter et al. 1967).

Rye and oats are both monocotyledons. Schenk and Hildebrandt (1972) reported that a high level of auxin-type growth regulating substances generally favored cell growth of monocotyledonous cultured in vitro, while low levels of cytokinins were essential for most dicotyledenous cell cultures. Some cellular cultures of dicotyledons adapted to a cytokinin-free medium containing a high level of 2,4-D or p-chlorophenoxyacetic acid (pCPA). They found that the preferred auxins were 2,4-D and pCPA.

Gregory et al. (1980) determined that callus development of winged bean (Psophocarpus tetragonolobus) occurred on media with 0.2 mg l of benzyl amino purine (BAP) and also on media with 0.2 mg l of naphthaleneacetic acid

(NAA) and 5 mg l BAP. A variation in response was observed depending on the hormonal combination. For example, on a medium with 2 mg l NAA and 0.02 mg l BAP, there was prolific root formation and very little production of callus. In the same study, Gregory et al. (1980) found NAA was effective for induction of callus, but NAA was not useful in media used to regenerate plants. Initiation of buds with NAA was rare. Bud initiation, however, did occur with indole acetic acid (IAA) in various combinations with BAP. When NAA was used, large quantities of callus tended to be produced.

Kartha et al. (1981) found that meristems of chickpea differentiated readily into shoots, either on modified Murashige-Skoog (MS) medium supplemented with benzyladenine (BA) alone or with combinations of BA and NAA. At high levels of BA (10 uM), the meristems differentiated into multiple buds. Their further growth, however, was suppressed. At 0.1 to 1 uM levels, BA induced excellent regeneration of shoot with profuse axillary branching from the main shoots. Morphogenetic responses identical to those obtained with 0.5 and 1 uM BA also were observed with combinations of BA and NAA. There was little difference in the morphogenetic response of meristems under different cytokinin:auxin ratios. Sharma et al. (1979) described the method for the establishment of callus culture and regeneration of whole plants in four Cicer species.

In bean (Phaseolus vulgaris L.), extract from seeds was required for regeneration of plants (Crocomo et al. , 1976), while in pigeon pea (Cajanus cajan) 5 Krad of gamma irradiation was used to obtain plant regeneration (Sharma Rao and Narayanaswamy 1975). Wild soybean (Glycine spp) cannot yet be routinely regenerated in vitro (Kameya and Widholm, 1981). However, shoot primordia have been obtained from soybean (Glycine max L.) hypocotyls (Kimball and Bingham, 1973; Oswald et al., 1977).

The hormonal requirements are not specific for forage legumes. For

example, hormones are not required for plant regeneration in alfalfa (Walker et al., 1979). But a wide range of concentrations of various hormones has resulted in regeneration of red clover (Trifolium pratense). The source of explant also can affect the frequency of regeneration of forage legumes. In red clover, the frequency of plant regeneration was 1% from cotyledons and 30 - 80 % from meristematic tissue (Phillips and Collins, 1979).

The season of the year can affect callus initiation from explants, especially if the donor plant is field grown. Seasonal variations in the concentration of endogenous auxins have been observed (Wodzicki, 1978). The establishment of potato (Solanum tuberosum L.) cultures has been reported to be dependent upon the season (Mellor and Stace-Smith, 1969; Harvey and Grasham, 1969). For potato, spring and summer were found to be the optimum seasons for starting cultures.

Genotypical differences have been observed in plant regeneration. Of 14 genetic lines of pea (Pisum sativum L.) tested for regeneration, six lines could be regenerated after six months in vitro (Melmberg, 1979). Differences in plant regeneration also were reported for five cultivars of red clover (Phillips and Collins, 1979) and nine cultivars of alfalfa (Bingham et al., 1975).

Major gene effects were responsible for callus formation for maize (Zea mays L.) endosperm (Tabata and Motoyishi 1965). Izhar and Power (1977) noted that only a few genes may be involved in gene-hormone interactions, necessary for growth of petunia (Petunia spp.) from protoplasts. Plant regeneration from Medicago sativa L. callus cultures was highly heritable (Bingham et al., 1975). In standard alfalfa cultivars, regeneration was increased from 10 to 67% after two cycles of recurrent selection.

In summary, many crops have been studied in tissue culture. But only two studies (Sharma et al., 1979, Kartha et al., 1981) have examined

the tissue-culture requirements of chickpea. The need exists, therefore, to determine the composition of media that permit growth and differentiation of chickpea in tissue culture.

MATERIALS AND METHODS

Six cultivars of chickpea (Cicer arietinum L.) species were used in this study. Five cultivars: NEC 1163 (desi type identified by Cubero (1975) as microcarpa which has small brown seeds), ILC 202 (tall cultivar), ILC 262 (spring cultivar), ILC 482 (winter cultivar), and ILC 511 (large seeded type) were supplied from the International Center for Agricultural Research in the Dry Areas (Aleppo, Syria) and one cultivar (local) from Tunisia. Seeds were surface sterilized by submerging them for 15 seconds in 70% ethanol then placed in 5% commercial bleach solution for 15 minutes, rinsed twice in sterile distilled water, then soaked for 24 hours in sterile distilled water. The seed coat was removed and the seeds were germinated in test tubes containing nutrient - agar medium with modified Hoagland solution (Hoagland and Arnon, 1950; Johnson et al., 1957; Harper and Paulsen, 1969a, b; Table 1) plus 0.6% agar and 0.5% sucrose. The test tubes were capped and sealed with parafilm. All operations were done in a laminar flow hood. All the media were solidified by adding 0.6% Difco agar and were autoclaved at 121 C under 103.33 kPa pressure for 20 minutes. The pH was adjusted to 5.5 with sodium hydroxide and hydrochloric acid. The test tubes were placed in dark at room temperature for three days then placed in a growth chamber with an 18 hour photoperiod and a constant temperature of 25±1 °C. When the seedlings reached 3 to 4 cm length, sections of the stem (0.5 cm in length) were removed and placed in different media for callus initiation.

Effect of media DM1 and B5-1 on callus induction

Excised stems from young seedlings of the six cultivars were plated on petri dishes containing DM1 medium (Liang et al., 1982) containing the

basic DM medium (Table 2) plus 1 mg liter of IAA and on B5-1 containing the basic B5 (Gamborg et al., 1968) medium (Table 3) plus 1 mg liter of benzyl amino purine (BAP) plus 1 mg liter of IAA. Approximately 700 explants were used.

Effect of eight growth media on callus growth

Three cultivars [NEC 1163, ILC 262, and a local (Tunisia)] were used for this study. Excised stems were plated on B5-2 medium (medium B5, Table 3, plus 1 mg liter of BAP and 1 mg liter of 2,4-D). About 10 petri dishes were plated with each cultivar. The petri dishes were sealed with parafilm and incubated in the growth chamber with an 18 hour photoperiod at 25 C±1 C. The explants which produced calli were subcultured in a fresh B5-2 medium to allow further growth of the calli. When the calli were large enough, about 1 cm in diameter, the remaining part of the stem was removed, and the calli were divided into small pieces . After 40 to 60 days in culture, there were enough calli from each cultivar.

The media used were:

B5a B5 + 0.5 mg of 2,4-D liter + 1 mg of BAP liter

B5b B5 + 0.5 mg of IAA liter + 1 mg of BAP liter

B5c B5 + 0.5 mg of NAA liter + 1 mg of BAP liter

B5d B5 + 0.75 mg of 2,4-D liter + 1.5 mg of BAP liter

B5e B5 + 1 mg of BAP liter + 1 mg of 2,4-D liter

B5f B5 + 2 mg of 2,4-D liter + 1 mg of kinetin liter

B5g B5 + 4 mg of 2,4-D liter + 1 mg of kinetin liter

B5h B5 + 1 mg of 2,4-D liter + 1 mg of kinetin liter

Four calli of about the same size from each cultivar were plated onto each medium. The calli were weighed with a Sartorius balance type 1212MP with a sensitivity of 0.01g. The average weight of each callus was

about 0.05 g at the beginning of the culture. The four calli were plated into 100x15 mm petri dish and were sealed with parafilm. They then were incubated for 30 days in a growth chamber and then weighed and subcultured into a new, fresh medium for another 30 days. Each callus was weighed three times and subcultured three times. The treatments were assigned according to a split-plot design with the main plot containing the variety x medium treatment combinations and with the subplot consisting of the repeated measurements through time.

Regeneration attempt

Three explants were used (stem, leaf, and petiole) as sources for callus initiation. The calli were regenerated on B5-2 medium (medium B5, Table 3, plus 1 mg liter of BAP and 1 mg liter of 2,4-D). Then three media were used to determine the optimum conditions for plant regeneration from callus.

B5i 2.2 mg of zeatin liter + 1 mg of IAA liter

B5j 3 mg of kinetin liter + 1 mg of 2,4-D liter

DMG1 1 mg of kinetin liter + 1 mg of GA liter

The calli were grown at 25±1 °C under a 16 hour photoperiod from a light source composed of 20 W General Electric cool-white fluorescent lights.

RESULTS AND DISCUSSION

Effect of media DM1 and B5-1 on callus induction:

None of the explants excised from the six cultivars produced calli on DM1. The explants remained green for about 3 to 4 weeks and then turned brown and died. However, on modified B5 medium, a few calli were initiated, mainly by the following cultivars: NEC1163, ILC262, and local. These cultivars produced, respectively, 14.28%, 12.69%, and 10.20% calli from the incubated explants (Table 4).

For callus initiation, probably BAP had a more positive effect on callus induction compared to kinetin. Other cultivars may require different concentrations of auxin and cytokinin to induce callus formation. These results agree with Melmberg (1979) who found that intervarietal differences exist between lines of Pisum sativum tested for callus regeneration.

Effect of eight growth media on callus growth of three cultivars of chickpea:

The statistical analysis showed that the three cultivars responded differently to the eight growth media and the differences were significant (Table 5 , 6, and Fig 1). The mean fresh-weight gain of the cultivar ILC262 differed significantly from NEC1163 and local (Table 7). However, the latter were not significantly different from each other. The average growth of the three cultivars was higher under B5c medium, a medium which had 0.5 mg per liter of NAA and 1 mg per liter of BAP (Fig 2). An increase of 2,4-D in the media had a negative effect on callus growth (Table 6 and Fig 2). The culture time was significant (Table 5 and 7). The growth rate of the calli was fast during the first month (119%) compared to the second month (61%); the difference might have been due to the age of the calli (Fig 3).

The mean fresh weight of the calli was higher under B5c (0.1878 g) (Table 6, Fig 2) than under B5g, which gave the lowest mean fresh weight (0.0831 g). The rapid growth under B5c might have been due to the best combination of BAP and NAA, whereas the lowest mean was probably due to a high level of 2,4-D, which inhibited callus growth.

Regeneration attempt:

Regeneration is a requirement for a well-defined tissue culture system. Regeneration experiments were started as soon as calli had been established from the callus. The calli proliferated rapidly through the first subculture and then began to decline. Growth was very slow in B5i media in which the calli remained green for about three months and then turned brown. In medium B5j, the callus remained green for a longer period of time (4-5 months) without any shoot or root initiation. In DMG1, however, the calli did not survive more than 1 or 2 months and then they turned brown. Root formation was observed on callus regenerated from leaf explants in B5f medium but no shoot was initiated. Formation of roots in legumes has been observed by Gregory et al. (1980). Another medium was used as described by Sharma et al. (1979), but no root or shoot was observed. These findings do not support their earlier observations of shoot and root formation from calli of Cicer spp. exposed to a specific photoperiod after six weeks. Cultivar differences could explain the discrepancy between the present results and those previously cited. At present it does not appear that shoot development will occur in the absence of phytohormones. Further work on plant regeneration in chickpea is needed.

Table 1. Composition of modified Hoagland's solution.

Component	mg/l
KNO_3	505.5
$Ca(NO_3)_2 \cdot 4H_2O$	1,181.0
$MgSO_4 \cdot 7H_2O$	493.0
KH_2PO_4	68.05
$FeSO_4$	2.78
Na_2EDTA	3.78
KCl	3.728
H_3BO_3	1.546
$MnSO_4 \cdot H_2O$	0.845
$ZnSO_4 \cdot 7H_2O$	0.575
$CuSO_4 \cdot 5H_2O$	0.125
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0184

Table 2. Composition of DM medium.

Component	mg/l
KNO_3	2,500
NH_4NO_3	1,000
$CaCl_2.2H_2O$	300
$MgSO_4.7H_2O$	300
KH_2PO_4	340
$FeSO_4.7H_2O$	27.8
Na_2EDTA	37.3
$MnSO_4.H_2O$	15.5
$ZnSO_4.7H_2O$	8.5
H_3BO_3	6.2
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
KI	0.830
$Na_2MoO_4.2H_2O$	0.250
Thiamine.HCl	1.0
Pyridoxine.HCl	5.0
Nicotinic acid	5.0
Myo-inositol	100.0
Kinetin	2.0
Glycine	20.0
Lactalbumin hydrolysate	500.0
Sucrose	30,000.0

Table 3. Composition of B5 medium

Component	mg/l
$NaH_2PO_4.H_2O$	150
KNO_3	2,500
$(NH_4)_2SO_4$	134
$CaCl_2.2H_2O$	150
$MgSO_4.7H_2O$	250
KI	0.75
H_3BO_3	3.0
$MnSO_4.H_2O$	10
$ZnSO_4.7H_2O$	2.0
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
$FeSO_4.7H_2O$	27.8
Na_2EDTA	37.3
Nicotinic acid	1.0
Thiamine.HCl	10
Pyridoxine.HCl	1.0
Myo-inositol	100
Sucrose	20,000

Table 4. Frequency of callus induction on B5-1 medium.

Cultivars	Number of explants inoculated	Number of calli induced*	%
NEC1163	42	6	14.29
ILC202	48	1	2.08
ILC482	47	0	0
ILC262	63	8	12.70
ILC511	61	2	3.28
Local (TN)	49	5	10.20

* Contaminated calli were discarded and not included in this table.

Table 5. Analysis of variance of callus growth on different media.

Source of variance	d.f.	MS	F
Cultivars (C)	2	0.0207	7.00*
Media (M)	7	0.0101	3.43*
C x M	14	0.0030	3.24*
Time (T)	2	0.1115	122.42*
C x T	4	0.0090	9.91*
M x T	14	0.0036	3.93*
Error	28	0.0009	

* The F-test indicates a significant effect at the 0.01 level of probability.

Table 6. Mean fresh weight of calli averaged over all cultivars on eight media.

Medium	Mean fresh weight over over all times (g)
B5a (0.5 mg 2,4-D/l + 1 mg BAP/l)	0.1178^{bc} *
B5b (0.5 mg IAA/l + 1 mg BAP/l)	0.1297^{bc}
B5c (0.5 mg NAA/l + 1 mg BAP/l)	0.1878^{a}
B5d (0.75 mg 2,4-D/l + 1.5 mg BAP/l)	0.1381^{ab}
B5e (1 mg 2,4-D/l + 1 mg BAP/l)	0.1214^{bc}
B5f (2 mg 2,4-D/l + 1 mg kinetin/l)	0.0958^{bc}
B5g (4 mg 2,4-D/l + 1 mg kinetin/l)	0.0831^{c}
B5h (1 mg 2,4-D/l + 1 mg kinetin/l)	0.0900^{bc}

LSD (0.05) = 0.0549

* Means followed by the same letter are not significantly different at 0.05 probability level according to Fisher's LSD test.

Table 7. Mean fresh weight averaged over all media for all cultivars at different time periods.

Time (days)	Mean fresh weight of all TRT (g)
0	0.0537^{c*}
30	0.1178^{b}
60	0.1899^{a}

LSD (.05) = 0.0178

* Means followed by the same letter are not significantly different at .05 probability level according to Fisher's LSD test.

Table 8. Callus mean fresh weight for three cultivars averaged over all media and time.

Cultivars	Variety mean fresh weight (g)
NEC1163	0.0944^{b*}
ILC262	0.1522^{a}
Local (TN)	0.1148^{b}

LSD (0.05) = 0.0336

* Means followed by the same letter are not significantly different at 0.05 probability level according to Fisher's LSD test.

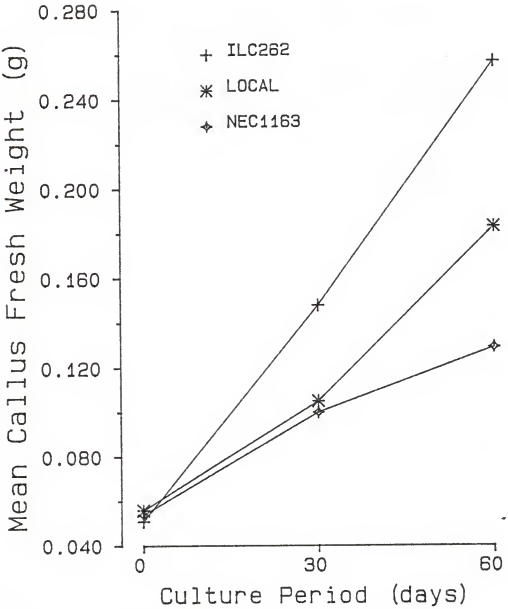

Figure 1. An increase of mean callus fresh weight for three cultivars over time.

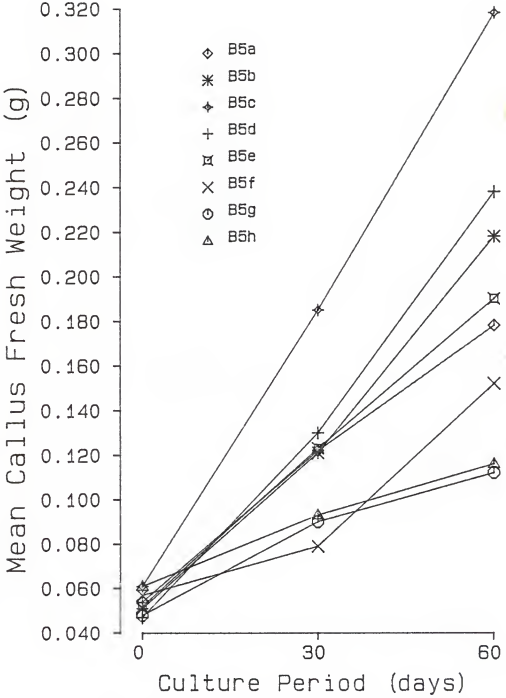

Figure.2 An increase of mean callus fresh weight on eight media over time.

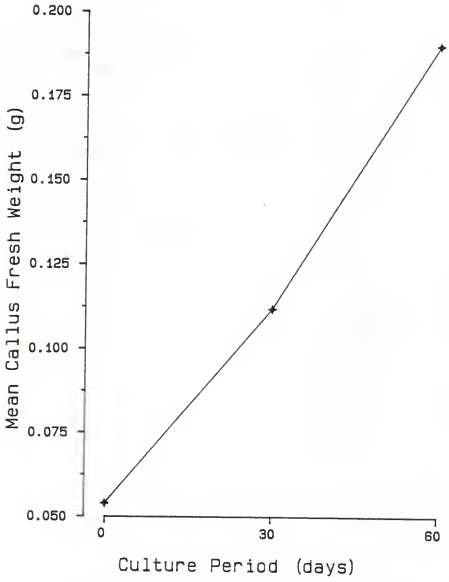

Figure 3. Mean callus fresh weight averaged over all media for all cultivars at different time periods.

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PART II

DROUGHT RESISTANCE OF CHICKPEA (*Cicer arietinum* L.)

ABSTRACT

Techniques for screening genotypes of chickpea (Cicer arietinum L.) were studied under growth chamber conditions. The techniques were based on seed germination and seedling growth using polyethylene glycol (PEG) with molecular weights of 600, 1,450, 3,350, and 8,000 as osmotica in aqueous media. Seedlings were grown in sand and soil by subjecting them to water stress until wilting. For the seed germination test and the experiment with PEG in solution culture, two culvars (ILC262 and ILC482) were used; however, for the experiment with sand or soil, six cultivars (NEC1163, ILC482, ILC202, ILC262, ILC511, and Local)were used. A germination rate index (GRI), plant height, plant fresh weight, and plant dry weight were determined in the study. The seeds were subjected to PEG at different concentrations for eight days, whereas seedlings were subjected to PEG for 14 days.

For the seed germination test, there was a significant PEG by cultivar interaction, and the two cultivars responded differently to different PEG molecular weights. ILC482 had the highest GRI under all PEG concentrations, except for PEG 8,000 (17%), with which no seeds of either cultivar germinated.

For the case of seedling growth with PEG solutions, there was no significant difference between the two cultivars. Plant height showed some difference, but differences were not consistent from one PEG concentration to another, as was seen on the seed germination test. Leaves were damaged, apparently from the use of PEG.

The use of sand or soil did not give any significant differences between the two cultivars, which did differ by using PEG. But there were

differences among the six cultivars when grown in sand or soil. Since with the use of PEG there were differences between the two cultivars, the use of PEG as a screening tool may be possible for identifying drought-resistant cultivars. But further studies concerning the phytotoxicity of PEG are needed.

INTRODUCTION

Water is the most abundant component of living plants. Under field conditions, however, water supply may at times be insufficient for satisfactory plant development. An alternate strategy could be to improve crops genetically through breeding cultivars capable of withstanding low moisture stress.

The first phase of a breeding program for more efficient water use in plants will be to survey available germplasm and to identify both tolerant (resistant) and susceptible genotypes. Unfortunately, many traits that may be associated with efficiency of water use are complex and will require more difficult selective procedures. The most efficient selection procedures will be those where large numbers (at least a thousand per year) of plants can be rapidly evaluated at minimal cost. When possible, the use of seedlings in selection procedures can be useful. Screening for drought tolerance by withholding water from seedlings grown in soil has been reported for many crops.

The objective of this study was to compare the potential usefulness of polyethylene glycol (PEG) with molecular weights of 600, 1,450, 3,350, and 6,000 for simulating the influence of water stress upon germination and seedling growth in chickpea.

LITERATURE REVIEW

Water deficit alters the growth and development of plants (Hsiao 1973). Selection for yield in a drought stressed environment is predicted to be a more efficient selection strategy for yield improvement in dryland situations than selection in a more optimal environment (Richards 1978). Studies with oats (Avena sativa L.) indicate that selecting cultivars under low stress conditions is advantageous because the large genotypic variation will afford good genotypic differentiation of lines (Johnson and Frey 1967). However, genetic variance tends to be lower in stressed environments, making it more difficult to detect varietal differences (Richards 1978).

Relatively few reports are available in the literature of successful efforts to screen the seedlings of crop species for drought resistance under controlled environments. Hunter et al. (1936) were able to distinguish among eight inbred corn (Zea mays L.) lines with respect to drought resistance by observing response to simulated drought stress under laboratory conditions. Field data confirmed the validity of their procedure. Wright and Jordan (1970) were able to improve the drought resistance of Boer lovegrass (Eragrostis curvula Nees) by screening seedlings in a controlled environment, and subsequently combining the most resistant genotypes by appropriate plant breeding techniques.

Richards and Thurling (1979) found that seed germination may be a valuable criterion in establishing efficient procedures to select for stress tolerance and yield in dryland situations. Rumbaugh and Johnson (1981) found that alfalfa accessions that can emerge at -6.5 bars of soil water potential in the laboratory also have better field emergence and survival under drought compared to accessions that cannot emerge in the laboratory at

-6.5 bars.

Because of difficulties in maintaining satisfactory control of soil moisture, the validity of some of the field studies of plant-water relations is questionable. Water potentials obtained within the soil mass are at best only rough measurements of water potentials at root surfaces, according to Wiggans and Gardner (1959). The use of osmotic substrates to provide controlled water potentials around the roots offers opportunities for bypassing many uncertainties in field studies. The technique of growing seedlings in hydroponic solution, made by Hoagland and Arnon (1950), facilitates screening large numbers of cultivars and eliminates several problems encountered when growing seedlings in soil.

Polyethylene glycols (PEG) are neutral polymers available in a range of molecular weights, highly soluble in water and with low toxicity to mammals (Merck Index, 7th edn, 1960). Because of their properties, they have been used by several investigators to impose water stress on plants by decreasing the water potential of the rooting medium and, thus, the water potential of the plant (Cheesman et al., 1965; Kaufmann, 1969; Parmar and Moore, 1966; Tadmor et al., 1969; Thill et al., 1979; Young et al., 1977). Singh and Rai (1980) screened two varieties of chickpea (Cicer arietinum L.) for their comparative resistance or susceptibility to a given water stress by using PEG 6,000. They found water stress inhibited the growth of both cultivars, and the inhibition increased with increasing level of water stress. However, researchers have reported that PEG has toxic effects on plants (Jackson, 1962; Leshem, 1966). Lagerwerff et al.(1961) attributed PEG 6,000 toxicity to associated heavy metals and recommended dialysis or passage through ion exchange columns to remove these impurities. Lawlor (1970) suggested that PEG 1,000, 4,000, and 20,000 caused plant desiccation by blocking pathways of water movement. The time and duration of applying

the stress treatment are critical factors to be considered when establishing a screening procedure for drought tolerance. Screening during vegetative development may provide a measure of stress on cell enlargement and leaf area development, but would not provide information on stress as it affects photosynthetic rates during seed development or the effects on pod and flower abortion. Differential responses of wheat seedlings to water stress was best evaluated past the stage of the first leaf emergence (Blum et al. 1980). Sullivan and Ross (1979) found that sorghum was more susceptible to drought during flowering than during the vegetative stage. If it is possible to detect genetic variability at an earlier stage than flowering, then the evaluation of large populations could be handled in a shorter period of time. There is no evidence whether drought tolerance at the seedling stage correlates with results obtained at later periods of vegetative growth. Kilen and Andrew (1969) and Williams et al. (1967) indicated that the two stages correlate, while Blum et al. (1980) showed that drought resistance in the germinating seed and developing seedling is process-specific.

MATERIALS AND METHODS

Germination test

Two chickpea cultivars ILC262 (spring cultivar) and ILC482 (winter cultivar) were used for this study. Ten seeds of each cultivar were germinated on three layers of towel paper in 100x12 mm glass petri dishes with polyethylene glycol (PEG) solution. PEG molecular weights of 600, 1,450, 3,350, and 8,000 were used. Two concentrations from each PEG were used to establish different solutions: 5 and 10% from PEG 600; 11 and 14% from PEG 1,450; 13 and 16% from PEG 3,350; and 14 and 17% from PEG 8,000. These solutions were prepared by dissolving the appropriate amount of PEG 600 and 1,450 (vol. vol.) or 3,350 and 8,000 (wt. vol.) in distilled water. The solute-potential was approximated from the graphs of Lawlor (1970). The solute-potential was also measured using thermocouple psychrometers. Three replications (petri dishes) of each treatment were placed at room temperature with an 14 hour photoperiod on a laboratory bench for eight days. Results were expressed in terms of germination rate index (GRI). Germination was scored when the root was initiated and the embryo axis reached a length of 5 mm. To consider the slow germination of seeds, George's mathematical weighting procedure (1967) was modified and computed for chickpea as follow:

GRI = (% germination at day two) + 0.75(% germination at day four) + 0.50(% germination at day six) + 0.25(% germination at day eight)

The experimental design was a completely randomized design with three replications.

Seedling growth

Experiment A: Two chickpea cultivars, ILC 262 and ILC 482, were used for this study to compare the usefulness of polyethylene glycol as an osmotic agent. Polyethylene glycol with molecular weights of 600, 1,450, 3,350, and 8,000 were studied. The seeds were germinated in vermiculite. When seedlings reached 2 to 3 cm in height, they were transferred to 2 liter black plastic pots, 16 cm in diameter, where they were grown hydroponically. In the growth chamber, day night temperatures were maintained at 25/20 °C with a 14 hour photoperiod. Illumination was provided by fluorescent and incandescent bulbs 80cm above the plant canopy. Light intensity was approximately 700 micro Einsteins sec cm^2. The pots were covered with black plastic tops having 6 holes, 1.2cm in diameter, and spaced 3.5cm apart. The radicle was placed in the hole when the seedling was transplanted. Each seedling was supported with a cork and glass wool. Pots were aerated throughout the experiment by an air pump. All seedlings were grown in Hoagland's nutrient solution (Hoagland and Arnon, 1950; Johnson et al. 1957; Harper and Paulsen, 1969a b; Table 1) for one week for a preconditioning period. After the first week, as in the germination test, the treatments were initiated by using PEG with molecular weights of 600, 1,450, 3,350, and 8,000. The solute potential was approximated from the graphs of Lawlor (1970). Five ml of iron solution ($FeSO_4$-Tartrate: 0.6% $FeSO_4.7H_2O$ and 0.4% tartaric acid solution) were added weekly to each pot. The solutions were completely changed at seven-day intervals. Hoagland's solution was added as needed to the pots during the seven-day period. The pH of the solution was adjusted to 5.5 by sodium hydroxide and by hydrochloric acid. The seedlings were subjected to the PEG treatment for 14 days. Control plants were maintained in Hoagland's nutrient solution during the same period of time. After PEG treatment, the plants were removed from the growth chamber for the

following measurements:

1) Plant height (cm).

2) Fresh weight of shoots and leaves (g).

3) Dry weight of shoots and leaves (g), determined after drying in the oven at 70°C for 48 hours.

Experiment B: Six chickpea cultivars (Cicer arietinum L.) were used in this study. Five cultivars [NEC1163 (desi type identified by Cubero (1975) as microcarpa, which has small brown seeds), ILC202 (tall cultivar), ILC262 (spring cultivar), ILC482 (winter cultivar), and ILC511 (large seed type)] were supplied by ICARDA, and one cultivar (local) came from Tunisia. Seeds were germinated in vermiculite. When the seedlings reached 2 to 3 cm length, each seedling was transplanted into a 9.5x9.5 cm plastic pot filled with sterile sand. Pots were arranged in a growth chamber in a completely randomized design with three replications.

The plants were watered as needed for the first week using Hoagland's nutrient solution. Then the nutrient solution was discontinued for pots receiving the stress treatment, while the controls continued to be watered throughout the experiment. At the end of the experiment, the plants were harvested and the following measurements were made:

1) Plant height (cm).

2) Fresh weight of shoots and leaves (g).

3) Dry weight of shoots and leaves (g), determined after drying in the oven at 70°C for 48 hours.

The experimental design was a completely randomized design with three replications.

EXPERIMENT C: This experiment was the same as experiment B, except that a

mixture of 1 part soil: 1 part peat: 1 part sand was substituted for the sand. The data were analyzed in the same way as in experiment B.

RESULTS AND DISCUSSION

SEED GERMINATION TEST:

The cultivar ILC482 showed a higher germination rate index percent (GRI) than the cultivar ILC262 (Table 2). The analysis of variance for germination rate index (GRI) indicated that highly significant differences for PEG, cultivars, and PEG by cultivar existed (Table 3). There was a significant PEG by cultivar interaction indicating that the two cultivars responded differently to different PEG molecular weights. ILC262 showed a large decrease in GRI compared to ILC482 (Fig 1). ILC482 germinated (95.83 %) under PEG 600 (5%), while the germination rate of ILC262 was 38.75 %. At a higher molecular weight of PEG, the GRI decreased for both cultivars. This possibly indicated that PEG of higher molecular weight is more phytotoxic for seed germination than PEG of lower molecular weight (PEG 600). To be confident about the osmotic concentration of the PEG solution, a measurement of the solutions was made and the results are reported in Table 4. The results indicated essentially no differences from the values estimated from Lawlor's graphs (1970) for the different solutions except for the cases of PEG 600 and PEG 8,000. The response to the same osmoticum (PEG), but with different molecular weights, had different effects on the GRI (Table 5). This supports the findings of Jackson (1962) and Leshem (1966) who found phytotoxicity of PEG. Since some differences occurred even with the control, the cultivar differences observed may be in part related to the seed quality of the two cultivars.

SEEDLING GROWTH:

The results of seedling growth subjected to different PEG solutions are reported in Table 6. The analysis of variance for plant height, fresh

weight, and dry weight of seedlings subjected to moisture stress in nutrient solution are given in Table 7. Highly significant differences were found among the PEG levels. However, no differences were detected among the two cultivars (Table 7). The cultivar-by-PEG interaction was significant only for plant height. Cultivar-by-PEG interaction for fresh weight and dry weight was not significant,indicating that the two cultivars responded alike to the different PEG molecular weights. The plant height, fresh weight, and dry weight were reduced as the molecular weight of PEG increased. The reduction of plant height was significantly different between the control and PEG 600 (5%) and the rest of the treatments (Table 8). There were no significant differences between PEG 600 (8%), PEG 1,450 (11%), and PEG 3,350 (13%), when plant height was considered, but differences existed between [PEG 600 (8%) and PEG 1,450 (11%)] and [PEG 8,000 (17%) and PEG 3,350 (16%)]. Also the effect of PEG 600 (5%) on fresh weight was significantly different from the rest of the other PEG solutions. Thus PEG of lower molecular weight seemed to give more differences between the two cultivars than PEG of higher molecular weight.

SAND EXPERIMENT:

The results of this experiment are reported in Table 9. In this study, plant height was significantly reduced under stress treatment, while there was no significant difference for plant fresh weight or dry weight (Table 10 and 11). The non-significant interaction effect indicated that the six cultivars responded similarly under the control or stress treatment. The non-significance was probably due to the fact that the soil-water moisture decreased sharply in a short period of time; enough time was not allowed to distinguish among the cultivars' ability to survive under low moisture content.

SOIL EXPERIMENT:

Evaluation of the six cultivars for stress tolerance was also investigated by comparing plant height, plant fresh weight, and dry weight in soil (Table 12). There were significant differences among the six cultivars, indicating that the cultivars responded differently to the stress (Table 13). There were no significant differences of "cultivar-by-stress" for both height and dry weight except for fresh weight. The two cultivars (ILC462 and ILC282) studied using PEG for seed and seedling growth did not differ from each other in the sand and soil experiments (Table 14).

The reduction in the growth of Cicer arietinum L. with PEG as osmoticum was observed by Singh and Rai (1980), who studied two chickpea cultivars. They also found that with an increase in water stress inhibition of growth increased. They also found that changes in plant fresh weight were more affected than those of height or dry weight.

The results of these studies suggest that polyethylene glycol may be used to screen for tolerance to moisture stress in chickpea seedlings, if a suitable technique were found to avoid its phytotoxicity such as described by Zur (1967). However, further studies are needed to determine whether or not screening at the seedling stage is related to yield performance under drought conditions.

Table 1. Composition of modified Hoagland's solution.

Component	mg/l
KNO_3	505.5
$Ca(NO_3)_2.4H_2O$	1,181.0
$MgSO_4.7H_2O$	493.0
KH_2PO_4	68.05
$FeSO_4$	2.78
Na_2EDTA	3.72
KCl	3.728
H_3BO_3	1.546
$MnSO_4.H_2O$	0.845
$ZnSO_4.7H_2O$	0.575
$CuSO_4.5H_2O$	0.125
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.0184

Table 2. Effect of PEG solutions on seed germination of two chickpea cultivars.

Treatment	ILC262	ILC482
	%	
Control	73.75	100
PEG 600 (5%)	38.75	95.83
PEG 600 (8%)	38.75	80.83
PEG 1,450 (11%)	16.25	77.50
PEG 1,450 (14%)	5	11.67
PEG 3,350 (13%)	8.75	64.17
PEG 3,350 (16%)	0	7.50
PEG 8,000 (14%)	11.25	21.67
PEG 8,000 (17%)	0	0

Table 3. Analysis of variance for seed germination rate index (GRI).

Source of variance	d.f.	m.s.	f
PEG	8	5380.94	133.34**
Cultivar	1	9481.48	234.95**
PEG x Cultivar	8	727.37	18.02**
Error	27	40.35	

** The F-test indicates a significant effect at the 0.01 level of probability.

Table 4. PEG water potential measured by psychrometer.

PEG	Estimated water potential (bars)	Measured water potential (bars)
PEG 600 (5%)	-3	-1.67
PEG 600 (8%)	-5	-3.91
PEG 1,450 (11%)	-3	-3.27
PEG 1,450 (14%)	-5	-5.50
PEG 3,350 (13%)	-3	-3.59
PEG 3,350 (16%)	-5	-6.14
PEG 8,000 (14%)	-3	-1.67
PEG 8,000 (17%)	-5	-4.87

Table 5. Mean of the germination rate index over both cultivars on nine treatments.

Treatment	Germination rate index
Control	89.5[a] *
PEG 600 (5%)	73.0[b]
PEG 600 (8%)	64.0[c]
PEG 1,450 (11%)	53.0[d]
PEG 1,450 (14%)	9.0[g]
PEG 3,350 (13%)	42.0[e]
PEG 3,350 (16%)	4.5[gh]
PEG 8,000 (14%)	17.5[f]
PEG 8,000 (17%)	0.0[h]

LSD (0.05) = 8.24

* Means followed by the same letter are not significantly different from each other at the 0.05 probability level according to Fisher's LSD test.

Table 6. Effect of PEG solutions on seedling growth of two chickpea cultivars.

Treatment	Plant height (cm)		Fresh weight (g)		Dry weight (g)	
	ILC262	ILC482	ILC262	ILC482	ILC262	ILC482
Control	23.97	28.07	2.20	2.71	0.30	0.39
PEG 600 (5%)	24.20	22.17	1.98	1.73	0.35	0.34
PEG 600 (8%)	21.27	20.52	1.28	1.46	0.25	0.29
PEG 1,450 (11%)	20.08	20.75	1.08	1.23	0.38	0.22
PEG 1,450 (14%)	18.33	18.67	0.94	1.17	0.18	0.21
PEG 3,350 (13%)	21.18	18.27	1.25	0.82	0.23	0.17
PEG 3,350 (16%)	16.88	15.32	0.59	0.64	0.12	0.15
PEG 8,000 (14%)	19.70	17.30	1.03	0.99	0.21	0.18
PEG 8,000 (17%)	16.25	17.63	0.53	0.84	0.15	0.19

Table 7. Analysis of variance for seedling moisture stress.

Source of variance	d.f.	Plant height		Fresh weight		Dry weight	
		MS	F	MS	F	MS	F
PEG	8	114.65	19.73**	4.09	26.44**	0.070	4.37**
Cultivar	1	3.38	0.58	0.13	0.87	0.001	0.04
PEG x Cultivar	8	14.84	2.55*	0.26	1.68	0.021	0.95
Error	90	5.81		0.15		0.016	

*, ** The F-test indicates a significant effect at the 0.05 and 0.01 level of probability respectively.

Table 8. Mean height, fresh weight, and dry weight for seedling moisture stress in PEG.

Treatment	Height (cm)	Fresh weight (g)	Dry weight (g)
Control	26.017^{a*}	2.455^{a}	0.344^{a}
PEG 600 (5%)	23.183^{b}	1.853^{b}	0.344^{a}
PEG 600 (8%)	20.892^{c}	1.373^{c}	0.272^{abc}
PEG 1,450 (11%)	20.417^{cd}	1.154^{cd}	0.300^{ab}
PEG 1,450 (14%)	18.500^{de}	1.054^{cd}	0.193^{cd}
PEG 3,350 (13%)	19.725^{cd}	1.036^{d}	0.201^{bcd}
PEG 3,350 (16%)	16.100^{f}	0.616^{f}	0.138^{d}
PEG 8,000 (14%)	18.500^{de}	0.872^{de}	0.192^{cd}
PEG 8,000 (17%)	16.942^{ef}	0.683^{ef}	0.173^{cd}
LSD (0.05)	1.955	0.319	0.103

* Means followed by the same letter in each column are not significantly different from each other at the 0.05 probability level according to Fisher's LSD test.

Table 9. Mean plant height, fresh weight, and dry weight of six chickpea cultivars grown under control and stress conditions using sand.

Cultivars	Plant height (cm)		Fresh weight (g)		Dry weight (g)	
	Control	Stress	Control	Stress	Control	Stress
NEC1163	33.70	29.57	3.07	1.05	0.58	0.39
ILC262	36.90	26.53	2.97	1.06	0.56	0.40
ILC262	30.10	25.13	3.05	0.98	0.56	0.36
ILC482	28.63	23.90	2.45	1.30	0.56	0.46
ILC511	24.53	21.47	2.76	0.91	0.54	0.37
Local (TN)	27.67	25.63	3.48	1.32	0.68	0.47

Table 10. Analysis of variance for seedling moisture stress in sand.

Source of variance	d.f.	Plant height		Fresh weight		Dry weight	
		MS	F	MS	F	MS	F
Cultivar	5	68.15	8.63**	0.28	0.74	0.013	1.47
Stress	1	214.13	27.11**	31.25	101.60**	0.260	31.40**
Cultivar x Stress	5	12.62	1.60	0.21	0.67	0.002	0.29
Error	24	7.90		0.31		0.008	

** The F-test indicates a significant effect at the 0.01 level of probability.

Table 11. Mean plant height, fresh weight, and dry weight of six chickpea cultivars grown under control and stress conditions using sand.

Cultivars	Plant height (cm)		Fresh weight (g)		Dry weight (g)	
	Control	Stress	Control	Stress	Control	Stress
NEC1163	33.70^{ab}*	29.57^{a}	3.07^{ab}	1.05^{a}	0.58^{a}	0.39^{a}
ILC202	36.90^{a}	26.53^{ab}	2.97^{ab}	1.06^{a}	0.56^{a}	0.40^{a}
ILC262	30.10^{bc}	25.13^{abc}	3.05^{ab}	0.98^{a}	0.56^{a}	0.36^{a}
ILC482	28.63^{c}	23.90^{bc}	2.45^{b}	1.30^{a}	0.56^{a}	0.46^{a}
ILC511	24.53^{c}	21.47^{c}	2.76^{ab}	0.91^{a}	0.54^{a}	0.37^{a}
Local (TN)	27.67^{c}	25.63^{abc}	3.48^{a}	1.32^{a}	0.68^{a}	0.47^{a}
LSD (0.05)	4.51	4.51	0.89	0.89	0.15	0.15

* Means followed by the same letter in each column are not significantly different from each other at the 0.05 probability level according to Fisher's LSD test.

Table 12. Mean plant height, fresh weight, and dry weight of six chickpea cultivars grown under control and stress conditions using soil.

Cultivars	Plant height (cm)		Fresh weight (g)		Dry weight (g)	
	Control	Stress	Control	Stress	Control	Stress
NEC1163	37.83	27.50	3.48	0.80	0.67	0.36
ILC202	33.17	27.50	2.82	0.85	0.59	0.40
ILC262	28.00	22.17	4.21	0.91	0.83	0.45
ILC482	31.83	24.17	3.95	0.86	0.80	0.44
ILC511	32.50	20.50	3.76	0.78	0.75	0.37
Local (TN)	30.17	23.00	4.53	1.40	0.91	0.56

Table 13. Analysis of variance for seedling moisture stress in soil.

Source of variance	d.f.	Plant height		Fresh weight		Dry weight	
		MS	F	MS	F	MS	F
Cultivar	5	38.04	5.51**	0.89	13.18**	0.049	10.22**
Stress	1	676.00	97.44**	73.44	1083.92**	0.970	202.83**
Cultivar x Stress	5	7.92	1.15	0.35	5.18**	0.008	1.67
Error	24	6.90		0.07		0.005	

** The F-test indicates a significant effect at the 0.01 level of probability.

Table 14. Mean plant height, fresh weight, and dry weight of six chickpea cultivars grown under control and stress conditions using soil.

Cultivars	Plant height (cm)		Fresh weight (g)		Dry weight (g)	
	Control	Stress	Control	Stress	Control	Stress
NEC1163	37.83^{a} *	27.50^{a}	3.48^{d}	0.80^{b}	0.67^{c}	0.36^{b}
ILC202	33.17^{b}	27.50^{a}	2.82^{e}	0.85^{b}	0.59^{d}	0.40^{b}
ILC262	28.00^{b}	22.17^{bc}	4.21^{ab}	0.91^{b}	0.83^{ab}	0.45^{ab}
ILC482	31.83^{b}	24.17^{ab}	3.95^{bc}	0.86^{b}	0.80^{b}	0.44^{b}
ILC511	32.50^{b}	20.50^{c}	3.76^{cd}	0.78^{b}	0.75^{bc}	0.37^{b}
Local (TN)	30.17^{b}	23.00^{b}	4.53^{a}	1.40^{a}	0.91^{a}	0.56^{a}
LSD (0.05)	4.22	4.22	0.42	0.42	0.11	0.11

* Means followed by the same letter in each column are not significantly different from each other at the 0.05 probability level according to Fisher's LSD test.

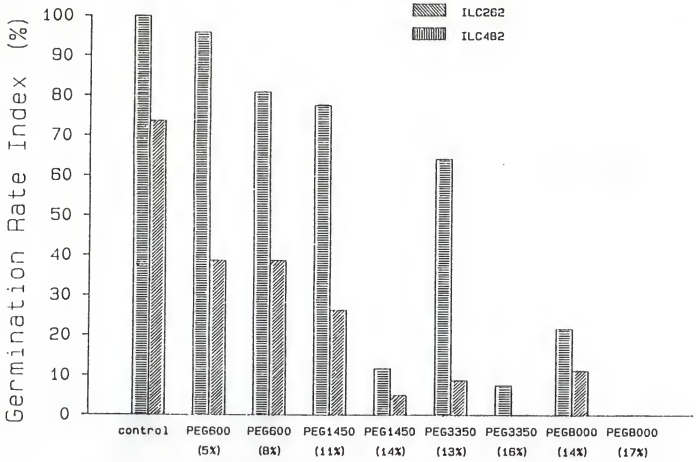

Figure.1 Germination rate index of two chickpea cultivars subjected to PEG.

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TISSUE CULTURE AND DROUGHT RESISTANCE OF CHICKPEA (*Cicer arietinum* L.)

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ABSTRACT

TISSUE CULTURE OF CHICKPEA

Tissue culture techniques were investigated using chickpea (Cicer arietinum L.) to ascertain requirements for callus formation and plant regeneration. Two media were used, DM1 and B5-1, to initiate callus from six cultivars: NEC1163, ILC202, ILC262, ILC482, ILC511, and Local (from Tunisia). Calli were produced mainly on the B5-1 medium from three cultivars: ILC262, NEC1163, and Local. Eight media were used (B5a, B5b, B5c, B5d, B5e, B5f, B5g, and B5h) to determine the suitability of growing calli from these three cultivars. The average callus fresh weight of the three cultivars was 0.188 g on B5c containing 1 mg of BAP/l and 0.5 mg of NAA/l, whereas the average callus fresh weight was only 0.083 g on B5g (4 mg of 2,4-D/l and 1 mg of kinetin/l). Among the three cultivars, ILC262 had the highest mean fresh weight of callus (0.152 g) over time on all media. The two other cultivars (NEC1163 and Local) did not differ significantly from each other (0.094 g and 0.115 g, respectively).

Three media were used for plant regeneration from calli (B5i, B5j, and DMG1). None of the media used for regeneration gave satisfactory results. Only root formation took place from leaf explants on B5f containing 2 mg of 2,4-D/l and 1 mg of kinetin/l which was used for callus growth.

DROUGHT RESISTANCE OF CHICKPEA

Techniques for screening genotypes of chickpea (Cicer arietinum L.) were studied under growth chamber conditions. The techniques were based on seed germination and seedling growth using polyethylene glycol (PEG) with molecular weights of 600, 1,450, 3,350, and 8,000 as osmotica in aqueous

media. Seedlings were grown in sand and soil by subjecting them to water stress until wilting. For the seed germination test and the experiment with PEG in solution culture, two culvars (ILC262 and ILC482) were used; however, for the experiment with sand or soil, six cultivars (NEC1163, ILC482, ILC202, ILC262, ILC511, and Local) were used. A germination rate index (GRI), plant height, plant fresh weight, and plant dry weight were determined in the study. The seeds were subjected to PEG at different concentrations for eight days, whereas seedlings were subjected to PEG for 14 days.

For the seed germination test, there was a significant PEG by cultivar interaction, and the two cultivars responded differently to different PEG molecular weights. ILC482 had the highest GRI under all PEG concentrations, except for PEG 8,000 (17%), with which no seeds of either cultivar germinated.

For the case of seedling growth with PEG solutions, there was no significant difference between the two cultivars. Plant height showed some difference, but differences were not consistent from one PEG concentration to another, as was seen on the seed germination test. Leaves were damaged, apparently from the use of PEG.

The use of sand or soil did not give any significant differences between the two cultivars, which did differ by using PEG. But there were differences among the six cultivars when grown in sand or soil. Since with the use of PEG there were differences between the two cultivars, the use of PEG as a screening tool may be possible for identifying drought-resistant cultivars. But further studies concerning the phytotoxicity of PEG are needed.